attacking species. For perbenzoate radicals, $\rho = -0.448$ as compared with -1.668 for *p*-chloroperbenzoate radicals.[11]

If the same species indeed serves as the hydrogen abstractor in each reaction, only bromine atoms could reasonably fill this role. Presumably the halogen arises from reaction of NBS with hydrogen bromide. Equations 7, 8 and 9 represent a likely chain-propagating sequence for the reaction. Step 8 is a fast, probably ionic, reaction which can be shown to occur essentially instantaneously at $-80°$ in toluene solution.

$$Br\cdot + RH \longrightarrow R\cdot + HBr \quad (7)$$

$$HBr + SNBr \longrightarrow SNH + Br_2 \quad (8)$$

$$Br_2 + R\cdot \longrightarrow RBr + Br\cdot \quad (9)$$

The process by which molecular bromine is formed initially in the reaction mixture is not specified, but many possible sources of this chain-carrying species can be postulated.

Two groups of workers, Sixma and Riem[3] and McGrath and Tedder,[4] have shown that at very low concentrations of molecular bromine allylic substitution, rather than addition to double bonds, may be the predominant reaction with olefins. The report[3] that NBS and bromine yield the same product mixtures in the bromination of two particular toluenes with a selectivity corresponding to a rho value of about -1.2 favors the mechanism involving molecular bromine. The indicated selectivity is, however, considerably less than that observed in the present work, a difference which is thought[17] to be too large to be explained on the basis of the change in solvent from carbon tetrachloride to benzene.

Low values for the selectivity of attack by bromine may arise as a result of the operation of the reverse reaction of hydrogen bromide with benzyl radicals,[13] a difficulty which we have avoided by greatly increasing the concentration of bromine relative to hydrogen bromide in the reaction mixture. Kooyman's selectivity ratios have been reproduced under conditions involving low Br_2/HBr ratios.

The conclusions which we have reached concerning the mechanism of benzylic bromination are not necessarily valid for the analogous allylic bromination reactions. Other free-radical reactions of NBS, notably its rearrangement to β-bromopropionyl isocyanate,[18] have been shown[19] to follow courses greatly influenced by the presence of traces of olefin. It is of interest, however, that a mechanistic scheme involving the three propagating steps (7, 8 and 9), which are postulated as being consistent with our results, can be reconciled with the over-all experimental rate law observed by Dauben for the allylic bromination of cyclohexene.

Continuing efforts in this Laboratory are designed to determine the relationship between brominations in the allylic and benzylic systems and to elucidate the nature of the chain initiating steps.

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THE NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS R. E. PEARSON
URBANA, ILLINOIS J. C. MARTIN

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(17) G. A. Russell, *Tetrahedron*, **8**, 101 (1960).

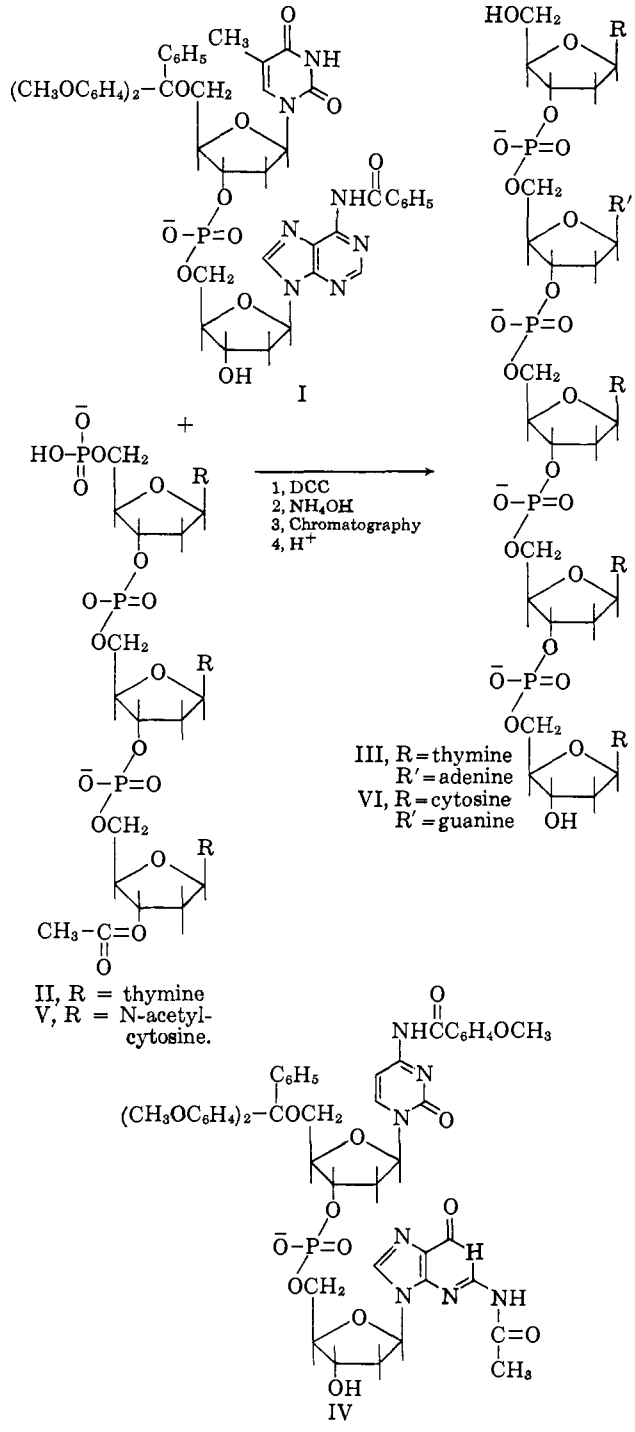
(18) J. C. Martin and P. D. Bartlett, *J. Am. Chem. Soc.*, **79**, 2533 (1957); H. W. Johnson and D. E. Bublitz, *ibid.*, **79**, 753 (1957).

(19) Unpublished work of Leslie Freiburg, Thomas Brownlee and Richard Panich.

THE SYNTHESIS OF DEOXYRIBO-POLYNUCLEOTIDES CONTAINING SPECIFIC NUCLEOTIDE SEQUENCES[1]

Sir:

Previously, methods have been reported for the preparation of suitably protected mononucleotides and for their polymerization to form homologous series of polynucleotides.[2] In the area of stepwise synthesis of oligonucleotides, studies have so far been carried out mostly with thymidine oligonucleotides[2-4] and the single synthetic experience with the mixed trinucleotide



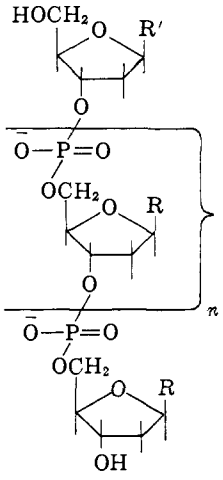
(1) This work has been supported by grants from the National Science Foundation, Washington, D. C., the National Cancer Institute of the National Institutes of Health and the Life Insurance Medical Research Fund, New York, N. Y.

(2) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, N. Y., 1961, Chapter 5.

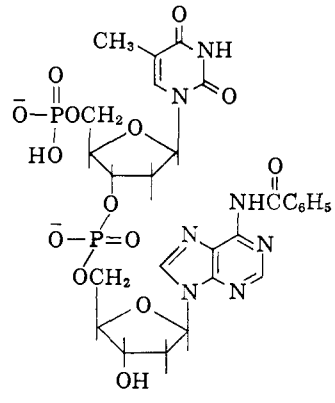
(3) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **81**, 4647 (1959).

(4) G. Weimann and H. G. Khorana, *ibid.*, **84**, 419 (1962).

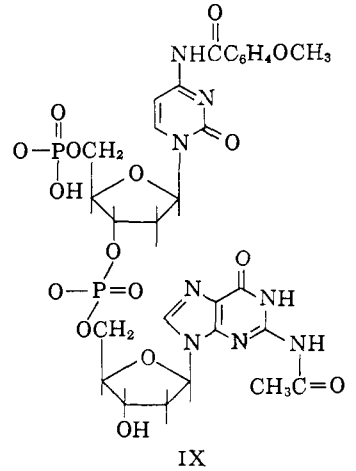
(d-TpApC)[5] emphasized the necessity for protecting the amino groups in the nucleosides.[3] The present communication outlines the approaches which have been developed successfully for the synthesis of oligo- and polynucleotides containing different nucleotides in predetermined sequences.



VII, R = cytosine
R′ = thymine



VIII



IX

Pentanucleotides.—5′-O-*p*-Methoxytritylthymidylyl-(3′→5′)-N-benzoyldeoxyadenosine (I) was prepared in 75% yield by condensation of N-benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate (0.7 mmole) with 5′-O-di-*p*-methoxytritylthymidine (1.5 mmoles) and a careful alkaline treatment. The thymidine trinucleotide (II) was prepared by acetylation of the trinucleotide (pTpTpT).[6] A mixture of pyridinium I (0.080 mmole), II (0.019 mmole), pyridinium Dowex-50 ion exchange resin (0.150 g.), dicyclohexylcarbodiimide (DCC) (250 mg.) in dry pyridine (0.5 ml.) and dimethylformamide (0.2 ml.) gave after 5 days at room temperature and subsequent chromatography on a DEAE-cellulose (carbonate) column the pentanucleotide (III) in 12% isolated yield. The latter was characterized by established chemical and enzymic methods.[3,4,6] In a parallel study, the condensation of N,O$^{3'}$-diacetyldeoxyguanosine-5′ phosphate with N-anisoyl-5′-O-di-*p*-methoxytrityldeoxycytidine[7] then a brief alkaline treatment gave IV which now was condensed with the protected deoxycytidine trinucleotide (V) by a procedure similar to that described above. After the removal of the protecting groups, pure deoxycytidylyl-(3′→5′)-deoxyguanylyl-(3′→5′)-deoxycytidylyl-(3′→5′)-deoxycytidylyl-(3′→5′)-deoxycytidine (VI) was isolated in 17% yield. The two examples cited demonstrate that suitable protecting groups are now available for the manipulation of all of the major deoxyribo-nucleosides and -nucleotides for polynucleotide synthesis.

Homologous Polynucleotides Bearing a Different Terminal Nucleoside.—A mixture of N,O$^{3'}$-diacetyldeoxycytidine-5′ phosphate and N-anisoyldeoxycytidine-5′ phosphate was polymerized as described previously.[8] After 9 days at room temperature, the mixture was treated in dry dimethylformamide with an excess of 5′-O-di-*p*-methoxytritylthymidine (0.75 mmole of the nucleoside for the polymeric mixture derived from a total of 0.7 mmole of the protected deoxycytidine nucleotide). Work-up after three days, including ammoniacal and acidic treatments, gave the homologous d-T-(pC)$_n$-pC (VII) as the new series of major products which were isolated pure and characterized. This method is complementary to that described earlier[6] for preparation of polynucleotides of the type d-pT(pT)$_n$-pC by copolymerizing N,O$^{3'}$-diacetyldeoxycytidine-5′ phosphate and thymidine-5′ phosphate. The present principle has been used in this Laboratory for the binding of deoxyribopolynucleotides to cellulose by ester bond formation with the hydroxyl groups of the latter.[9]

Deoxyadenylate-thymidylate and Deoxyguanylate-deoxycytidylate Copolymers.—*β*-Cyanoethyl thymidine-5′ phosphate was prepared by reaction of pyridinium thymidine-5′ phosphate with DCC in the presence of hydroacrylonitrile. The condensation of N-benzoyl-3′-O-acetyldeoxyadenosine-5′ phosphate (0.59 mmole) with *β*-cyanoethyl thymidine-5′ phosphate (0.88 mmole) in the presence of DCC (1.1 g.) and pyridine (1 ml.), a careful alkaline treatment and chromatography afforded the protected dinucleotide (VIII) in 31% yield. The analogous reaction of N,O$^{3'}$-diacetyldeoxyguanosine-5′ phosphate (4 mmoles) with N-anisoyldeoxycytidine-5′ *β*-cyanoethyl phosphate (2 mmoles) gave the protected dinucleotide (IX) (0.9 mmole). The protected dinucleotide (VIII) (0.5 mmole) was treated in dry pyridine (0.75 ml.) with DCC (2 mmoles) for 6 days at room temp. Work-up inclusive of an ammoniacal treatment and chromatography on a DEAE-cellulose column gave d-pTpApTpA (10.7%), d-pTpApTpApTpA (5%), d-pTpApTpApTpApTpA (2%) and higher homologs (1.1%), from which after treatment with bacterial alkaline phosphomonoesterase the polynucleotides d-T-(pApT)$_4$pA and d-T-(pApT)$_5$pA were isolated pure. The polymerization of the protected dinucleotide (IX) similarly gave the polynucleotides d-pCpGpCpG, d-pCpGpCpGpCpG and d-pCpGpCpGpCpGpCpG although the extent of polymerization was less satisfactory. The polynucleotides herein described are being used in studies of polynucleotide interactions as well as in enzymatic studies.

(5) For the system of abbreviations see Chapter 5, ref. 2.
(6) H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961).
(7) H. Schaller and H. G. Khorana, *Chem. and Industry*, 699 (1962).
(8) H. G. Khorana, A. F. Turner and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 686 (1961).
(9) P. T. Gilham, *ibid.*, **84**, 1311 (1962).

INSTITUTE FOR ENZYME RESEARCH
OF THE UNIVERSITY OF WISCONSIN
MADISON 6, WIS.
H. SCHALLER
G. WEIMANN
H. G. KHORANA

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ACYL-ENZYME INTERMEDIATES IN THE α-CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF "SPECIFIC" SUBSTRATES. THE RELATIVE RATES OF HYDROLYSIS OF ETHYL, METHYL AND *p*-NITROPHENYL ESTERS OF N-ACETYL-L-TRYPTOPHAN[1,2]

Sir:

The stepwise process for the catalysis of hydrolytic reactions by α-chymotrypsin (eq. 1) has received con-

(1) This research was supported by Grant H-5726 of the National Institutes of Health.
(2) Paper XVIII in the series, The Mechanism of Action of Proteolytic Enzymes.